

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

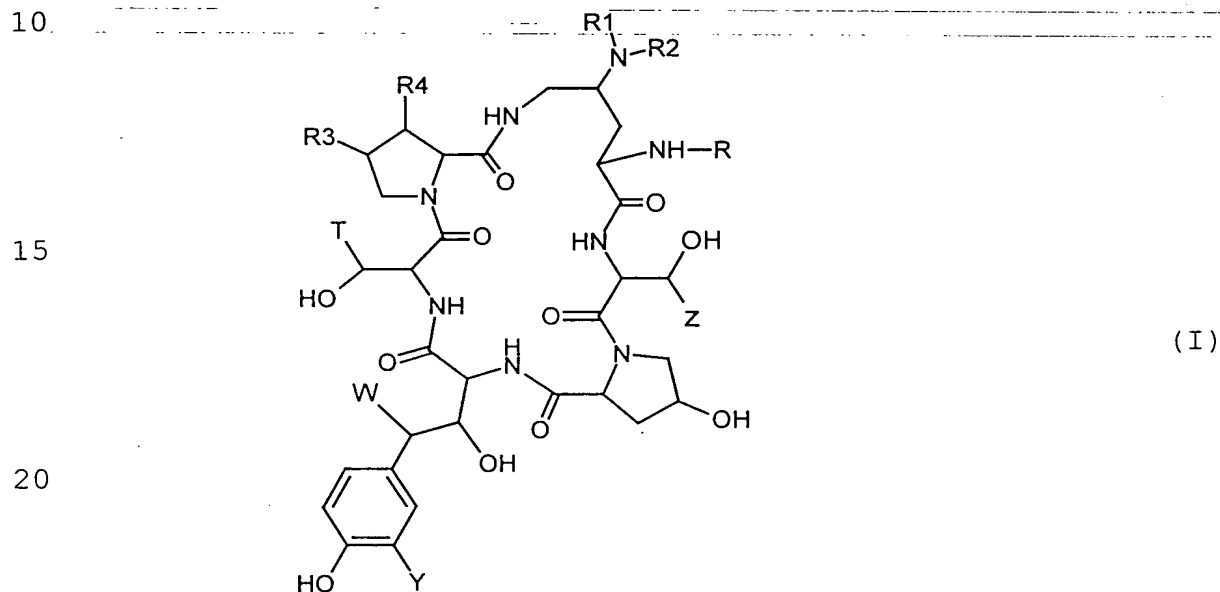
IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

New derivatives of echinocandine, their preparation process
and their use as antifungals.

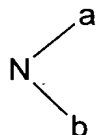
The present invention relates to new derivatives of
 5 echinocandine, their preparation process and their use as
 antifungals.

A subject of the invention is in all the possible isomer
 forms as well as their mixtures, the compounds of formula
 (I):



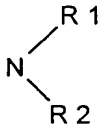
25 in which

either R_1 and R_2 identical to or different from one another,
 represent a hydrogen atom, a hydroxyl radical, a linear,
 branched or cyclic alkyl radical containing up to 8 carbon
 atoms optionally interrupted by an oxygen atom optionally
 30 substituted by a halogen atom, an OH radical, an

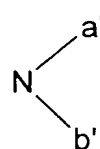


35 radical, a and b identical to or different from one another,
 representing a hydrogen atom or an alkyl radical containing
 up to 8 carbon atoms, a and b can optionally form with the
 nitrogen atom a heterocycle optionally containing one or more

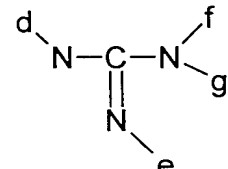
additional heteroatoms,
or R₁ forms with the endocyclic carbon atom

5 carrying the  radical a double bond and or R₂

represents an XRa radical, X representing an oxygen atom or
10 an NH or N-alkyl radical containing up to 8 carbon atoms and
Ra represents a hydrogen atom, a linear, branched or cyclic
alkyl radical containing up to 8 carbon atoms optionally
substituted by one or more halogen atoms, by one or more OH,
CO₂H, CO₂alk radicals, by an

15 

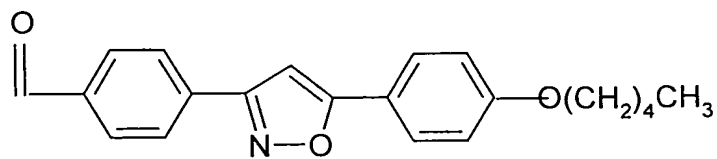
radical, a' and b' representing a hydrogen atom, an alkyl
20 radical containing up to 8 carbon atoms, a' and b' can form a
heterocycle optionally containing one or more additional
heteroatoms and/or by a heterocycle containing one or more
heteroatoms or R₂ represents a

25 

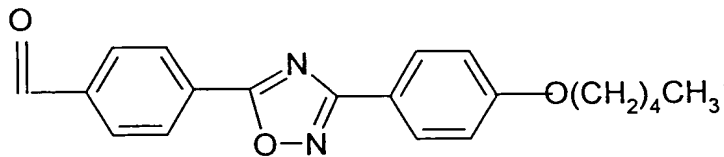
radical in which d, e, f and g represent a hydrogen atom or
30 an alkyl radical containing up to 8 carbon atoms, f and g can
moreover represent an acyl radical containing up to 8 carbon
atoms, e and f can also form a ring optionally containing one
or more heteroatoms,

R₃ represents a hydrogen atom, a methyl or hydroxyl radical
35 R₄ represents a hydrogen atom or a hydroxyl radical
R represents a radical chosen from the following radicals:

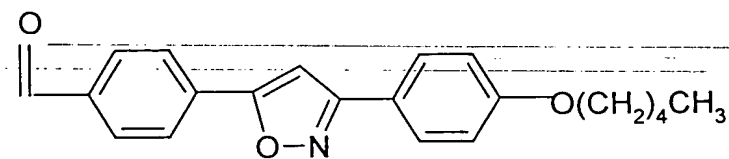
3



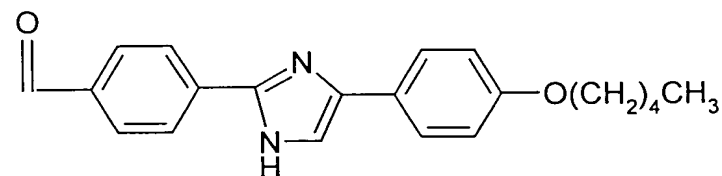
5



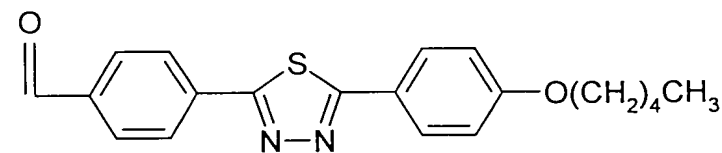
10



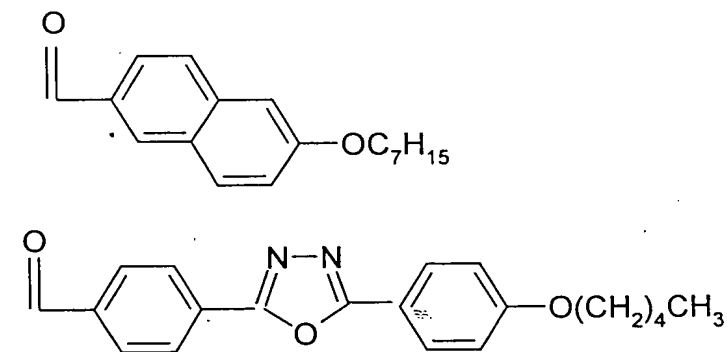
15



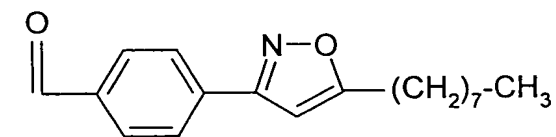
20



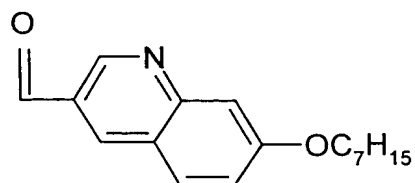
25

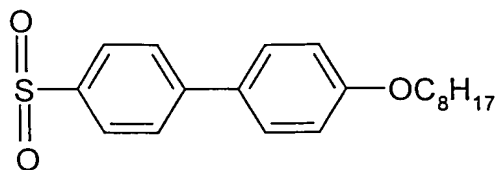


30



35





5

T represents a hydrogen atom, a methyl radical, a CH_2CONH_2 , $\text{CH}_2\text{C}\equiv\text{N}$ radical, a $(\text{CH}_2)_2\text{NH}_2$ or $(\text{CH}_2)_2\text{Nalk}^+\text{X}^-$ radical, X being a halogen atom and alk an alkyl radical containing up to 8 carbon atoms,

Y represents a hydrogen atom, a hydroxyl radical or a halogen atom or an OSO_3H radical or one of the salts of this radical,

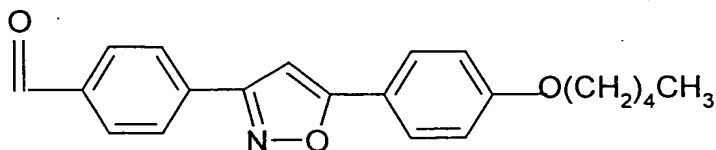
W represents a hydrogen atom or an OH radical,

15 Z represents a hydrogen atom or a methyl radical, as well as the addition salts with acids of the products of formula (I).

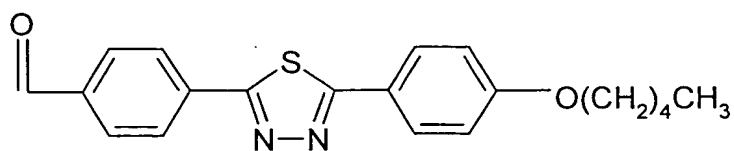
Among the addition salts with acids, there can be mentioned those formed with mineral acids, such as hydrochloric, hydrobromic, sulphuric or phosphoric acid or 20 with organic acids such as formic, acetic, trifluoroacetic, propionic, benzoic, maleic, fumaric, succinic, tartaric, citric, oxalic, glyoxylic and aspartic acids, alkanesulphonic acids, such as methane or ethane sulphononic acid, 25 arylsulphonic acids such as benzene or paratoluene sulphononic acids.

A more particular subject of the invention is the compounds of formula I in which T represents a hydrogen atom, those in which W represents a hydrogen atom, those in which Z 30 represents a methyl radical, those in which Y represents a hydrogen atom, those in which R_3 represents a methyl radical, those in which R_4 represents a hydroxyl radical and those in which R represents a

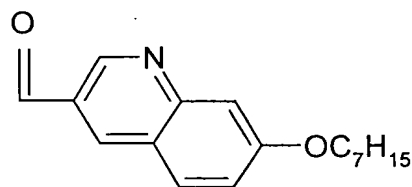
35



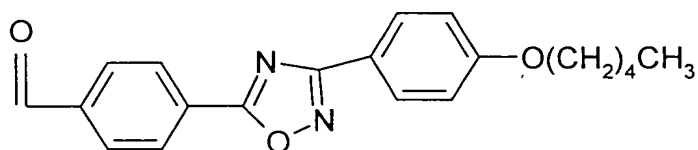
5



5



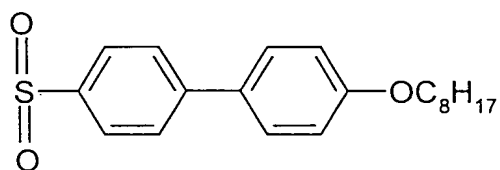
10



15

radical
or a

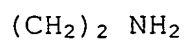
20



radical

those in which R_1 represents a hydrogen atom,

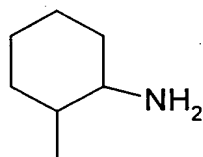
25 those in which R_2 represents a



radical

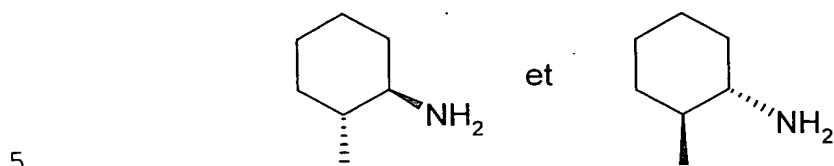
30 those in which R_2 represents a

35

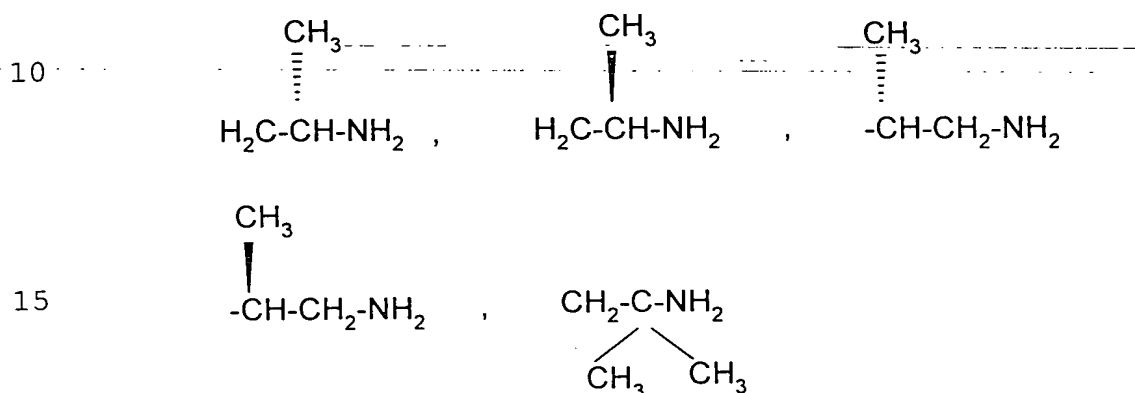


radical

and in particular the



radicals as well as those in which R₂ represents a



radical.

20 A more particular subject of the invention is the compounds of formula I the preparation of which is given hereafter in the experimental part.

The compounds of formula (I) have useful antifungal properties; they are in particular active on *Candida albicans*,
 25 and other *Candida* such as *Candida glabrata*, *krusei*, *tropicalis*, *pseudotropicalis*, *parapsilosis* and *Aspergillus fumigatus*, *Aspergillus flavus*, *Cryptococcus neoformans*.

The compounds of formula (I) can be used as medicaments in man or animals, in particular to combat invasive
 30 candidosis, digestive, urinary, vaginal or cutaneous candidosis, cryptococcosis, for example neuromeningeal, pulmonary or cutaneous cryptococcosis, bronchopulmonary and pulmonary aspergillosis and invasive aspergillosis in the immunosuppressed.

35 The compounds of the invention can also be used in the prevention of mycotic illnesses in the congenital or acquired immunosuppressed.

The compounds of the invention are not limited to a

pharmaceutical use, they can also be used as fungicides in fields other than the pharmaceutical field.

Therefore a subject of the invention is, as antifungal compounds, the compounds of formula (I) as well as their
5 addition salts with acids.

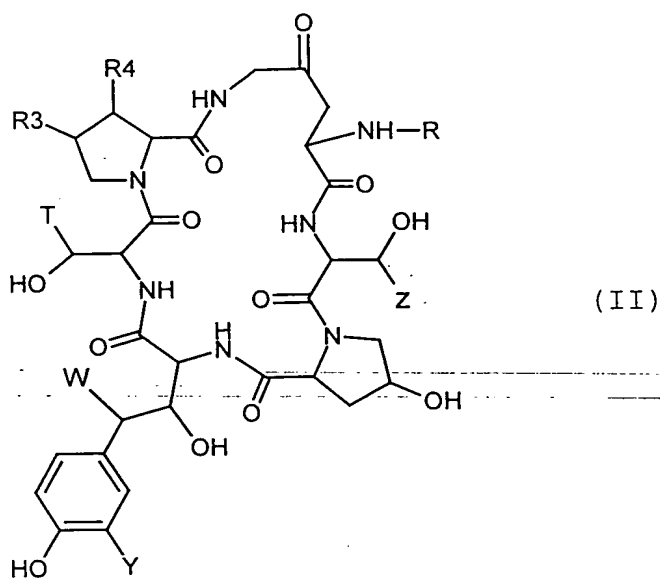
A subject of the invention is also the compounds of formula (I), as medicaments.

A most particular subject of the invention is the pharmaceutical compositions containing as active ingredient
10 ~~at least one compound of formula (I) or one of its addition~~ salts with pharmaceutically acceptable acids.

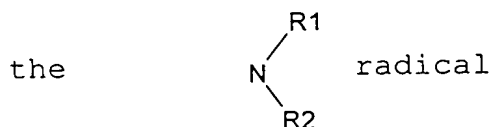
These compositions can be administered by oral, rectal, parenteral route or by local route as a topical application on the skin and mucous membranes, but the preferred routes
15 are the oral and parenteral routes.

They can be solid or liquid and can be presented in the pharmaceutical forms commonly used in human medicine, such as for example, plain or sugar-coated tablets, gelatin capsules, granules, suppositories, injectable preparations, ointments,
20 creams, gels; they are prepared according to the usual methods. The active ingredient or ingredients can be incorporated in the excipients usually used in these pharmaceutical compositions, such as talc, gum arabic, lactose, starch, magnesium stearate, cocoa butter, aqueous or
25 non-aqueous vehicles, fatty matter of animal or vegetable origin, paraffin derivatives, glycols, various wetting, dispersing or emulsifying agents, preservatives.

A subject of the invention is also a preparation process for the compounds of formula (I) characterized in that a
30 compound of formula (II).



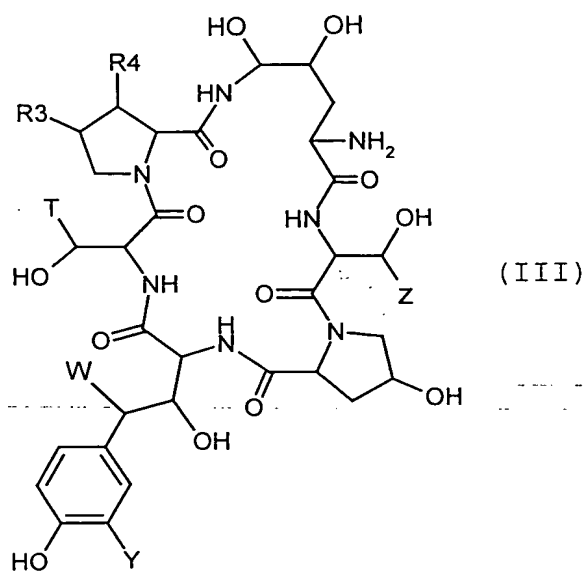
15 in which R, R₃, R₄, T, Y, W and Z retain their previous meaning, is subjected to the action of an amine or of an amine derivative capable of introducing



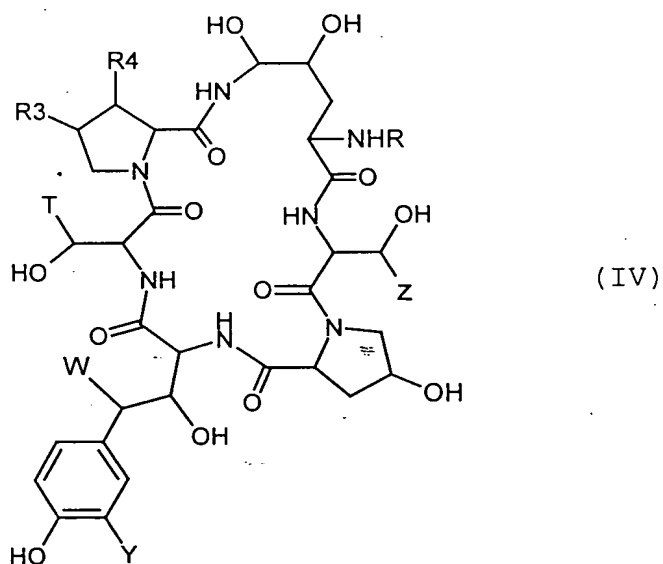
in which R₁ and R₂ retain their previous meaning and if
 25 desired to the action of a reducing agent,
 and/or of a functionalization agent of the amine,
 and/or of an acid in order to form the salt of the product
 obtained,
 and/or of a separation agent of the different isomers
 30 obtained,
 and in this way the compound of formula (I) as defined above
 is obtained.

The compounds of formula II used are new products and
 are in themselves a subject of the invention.

35 A subject of the invention is also a process
 characterized in that a compound of formula (III)

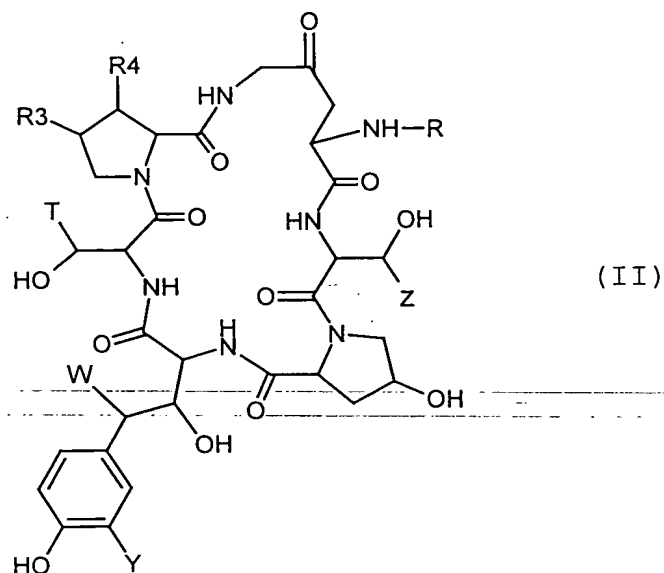


15 in which the different substituents retain their previous meaning is subjected to the action of an agent capable of replacing NH_2 by NHR , R retaining its previous meaning in order to obtain the compound of formula (IV)



35

which is subjected to the action of trimethylsilyl iodide in order to obtain the corresponding compound of formula (II)



The compounds of formula III and IV used are new products and are in themselves a subject of the present invention.

Among the preferred products of formula III and IV, there can be quite particularly mentioned the products the preparation of which is given hereafter in the experimental part.

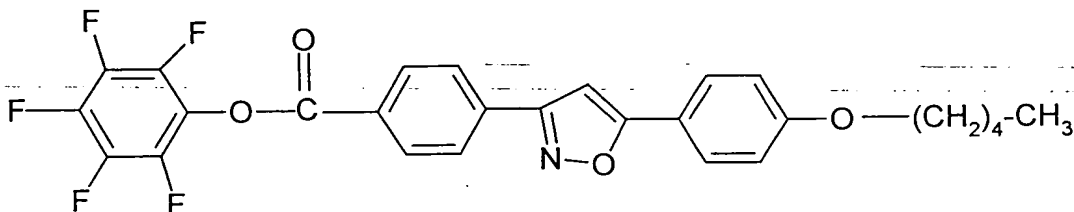
The following examples illustrate the invention without however limiting it:

Preparation 1: "nucleus" of deoxymulundocandine

2 g of deoxymulundocandine is dissolved in 20 ml of DMSO. This solution is poured into a suspension containing 120 g of *Actinoplanes utahensis* FH2264 in 870 ml of a KH₂PO₄, K₂HPO₄ buffer (pH: 6.8). The reaction mixture is maintained under agitation for 70 hours at 30°C. Filtration is carried out. The mycelium is washed with the phosphate buffer (pH: 6.8). The washing liquids and the filtrate are combined. The product obtained is chromatographed on a DIAION HP 20 resin and a product is obtained which is used as it is hereafter.

EXAMPLE 1: 1-[4-[(2-aminoethyl)amino]-N²-[[4-[5-[4-pentyl-oxy)-phenyl]-3-isoxazolyl]-phenyl]-carbonyl]-L-ornithine]-4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B (isomer A and isomer B).

10



15 Agitation is carried out for 29 hours, followed by filtering
and concentrating under reduced pressure. The residue is
taken up in ether, followed by triturating, separating,
washing with ethyl ether and chromatography on silica eluting
with a methylene chloride methanol mixture (85/15). In this
20 way the expected product is obtained $rf = 0.24$

STAGE B: 1-[4-oxo-N2-[[4-[5-[4-(pentyloxy)-phenyl]-isoxazol-3-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B.

6.12 ml of trimethylsilyl iodide is added to a
25 suspension containing 16.1 g of the product of Stage A and
374 ml of acetonitrile is added. Then the reaction medium is
heated for 15 minutes at 60°C followed by hydrolyzing with a
saturated solution of sodium thiosulphate. After bringing to
dryness under reduced pressure chromatography is carried out
30 on silica eluting with a methylene chloride, methanol, water
mixture 86-13-1. The sought product is obtained $rf = 0.23$.

Mass Spectrum

$$MH+ = 1083.6$$
$$M_{Na^+} = 1105.6$$

35 STAGE C: 1-[4-[(2-aminoethyl)-amino]-N2-[[4-[5-[4-(pentyloxy)-phenyl]-3-isoxazol-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate (Isomer A and isomer B).

8.6 mg of NaBH_3CN is introduced into a mixture of 120 mg of the product of the preceding stage, 2.4 ml of methanol, 60 mg of ethylenediamine diacetate in the presence of activated 4A siliporite. The reaction mixture is maintained under agitation and under a nitrogen atmosphere for 18 hours, followed by filtering and concentrating. The product obtained is purified by semi preparative HPLC eluting with an acetonitrile / H_2O /TFA mixture (40-60-0.02 %). 14.5 mg of sought product is recovered.

10 Mass spectrum

1127+ = MH^+

1149+ = Mna^+

The following are recovered: Isomer A: 14.5mg

Isomer B: 17.5 mg

15 **EXAMPLE 2:** *trans*-1-[4-[(2-aminocyclo-hexyl)-amino]-N2-[[4-[5-[4-(pentyloxy)-phenyl]-3-isoxazolyl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate (Isomer A and Isomer B).

Approximately 40 μl of acetic acid is added under agitation and under a nitrogen atmosphere to a solution containing 100 mg of the product obtained in Stage B of the preceding example 3 ml of methanol, 32 mg of (1R, 2R)(-)-1,2-diaminocyclohexane until a pH close to 6 is obtained, in the presence of activated 3A siliporite. Agitation is carried out for 5 minutes and 12 mg of NaBH_3CN is introduced. The reaction mixture is maintained under agitation for 18 hours. followed by filtering and concentrating under reduced pressure. The product obtained is purified by semi-preparative HPLC (eluent CH_3CN , H_2O , TFA 50-50-0.02 %).

30 Isomer A wt= 11 mg

Isomer B wt = 14 mg

Mass spectrum

1181.5 MH^+

35 **EXAMPLE 3:** *trans*-1-[4-[(2-aminocyclo-hexyl)-amino]-N2-[[4-[5-[4-(pentyloxy)-phenyl]-3-isoxazolyl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate (Isomer A and isomer B).

Operating as in Example 2 with (1S, 2S)-(-)-1,2-

diaminocyclohexane, the following are obtained:

Isomer A = 7.4 mg

Isomer B = 10.8 mg

Mass spectrum

5 1181.5 = MH+

EXAMPLE 4: 1-[4-[(2(S)-aminopropyl)-amino]-N2-[[4-[5-[4-(pentyloxy)-phenyl]-3-isoxazolyl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate (Isomer A and isomer B).

10 By operating as in Example 1 the following are obtained:

Isomer A: 13 mg

Isomer B: 10 mg

EXAMPLE 5: trans-1-[4-[(2-aminocyclo-hexyl)-amino]-N2-[[4-[3-[4-(pentyloxy)-phenyl]-1,2,4-oxadiazol-5-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate

15 **STAGE A:** 1-[(4R,5R)-4,5-dihydroxyN2-[[4-[3-[4-(pentyloxy)-phenyl]-1,2,4-oxadiazol-5-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B.

By operating as in Example 1 Stage A, the sought product is obtained.

Mass spectrum 1124 = MNa+

25 **STAGE B:** 1-[4-oxo-N2-[[4-[3-[4-(pentyloxy)-phenyl]-1,2,4-oxadiazol-5-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B.

By operating as in Example 1 Stage B, the sought product is obtained.

Mass spectrum 1106.6 = MNa+

30 1090.8 = MH+

STAGE C: trans-1-[4-[(2-aminocyclo-hexyl)-amino]-N2-[[4-[3-[4-(pentyloxy)-phenyl]-1,2,4-oxadiazol-5-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate.

35 By operating as in Example 1 Stage C, starting from 150 mg of the product of Stage B, and 51.4 mg of (1S,2S)1,2-diaminocyclohexane, 165 mg of crude product is obtained which is purified by semi preparative HPLC (KROMASIL C18 column)

(eluent: CH₃CN-H₂O-TFA 45-55-0.1).

The following are obtained: Isomer A 10.8 mg
Isomer B 5.2 mg

Mass spectrum: 1204 = MNa⁺

5 1182 = MHa⁺

EXAMPLE 6: 1-[4-[(2-aminoethyl)amino]-N₂-[[4-[5-[4-(pentyloxy)-phenyl]-1,3,4-thiadiazol-2-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate.

10 **STAGE A:** 1-[(4R,5R)-4,5-dihydroxy-N₂-[[4-[3-[4-(pentyloxy)-phenyl]-1,3,4-thiadiazol-2-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B.

A suspension containing 2 g of 4-[5-[4-(pentyloxy)-phenyl]-1,3,4-thiadiazol-2-yl]-benzoic acid, 30 ml of DMF and 30 ml of dioxan is agitated for 5 minutes at 20°C and 1.55 ml of tributylamine and 7.74 ml of isobutyl chloroformate are added at 0/±5°C. The reaction medium is agitated for 3 minutes at 0/±5°C then for 3 hours at ambient temperature.

20 4.53 g of the deoxymulundocandine nucleus obtained as in Preparation 1 is introduced. Agitation is carried out for 16 hours at 20°C, followed by concentrating to dryness. The residue is taken up in ethyl ether, followed by separating and washing with ethyl ether. After drying 7.8 g of product is obtained which is purified by chromatography on silica eluting with a methylene chloride-methanol-water mixture 86-13-1. 2.51 g of sought product is obtained.

STAGE B: 1-[4-oxo-N₂-[[4-[5-[4-(pentyloxy)-phenyl]-1,2,4-thiadiazol-2-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B.

30 By operating as in Stage B of Example 1, the sought product is obtained.

STAGE C: 1-[4-[(2-aminoethyl)amino]-N₂-[[4-[3-[4-(pentyl-oxy)-phenyl]-1,3,4-thiadiazol-2-yl]-phenyl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B.

35 By operating as in Example 1, Stage C starting from the product of the preceding stage and ethylenediamine diacetate,

the sought product is obtained.

Isomer A wt = 8 mg

Isomer B wt = 9 mg

EXAMPLE 7: trans 1-[4-[(2-aminocyclo-hexyl)-amino]-N2-[[4-[5-
5 [4-(pentyloxy)-phenyl]-1,3,4-thiadiazol-2-yl]-phenyl]-
carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-
L-serine-echinocandine B trifluoroacetate

By operating as in Example 1, starting from the product
of Stage B of Example 5 (50 mg) and (1S,2S)(+)1,2-
10 diaminocyclohexane (15.6 mg), the sought product is obtained.

Isomer A = 4 mg

Isomer B = 6.5 mg

EXAMPLE 8: trans 1-[4-[(2-aminocyclo-hexyl)-amino]-N2-[[4-[5-
[4-(pentyloxy)-phenyl]-1,2,4-thiadiazol-2-yl]-phenyl]-
15 carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-
L-serine-echinocandine B trifluoroacetate (Isomer A and
isomer B)

By operating as in Example 1 Stage C starting from the
product of Stage B of Example 5 (50 mg) and (1R,2R)-1,2-
20 diaminocyclohexane (15.6 mg), the sought product is obtained.

Isomer A = 8.8 mg

Isomer B = 10.6 mg

EXAMPLE: Pharmaceutical composition:

Tablets were prepared containing:

25 - Product of Example 1..... 150 mg
- Excipient s.q.f. 1 g
(Detail of excipient: starch, talc, magnesium stearate).

PHARMACOLOGICAL STUDY

30

A - Inhibition of the glucan synthase of *Candida albicans*.

Candida albicans membranes were purified according to
the process described by Tang et al Antimicrob. Agents
Chemother 35, 99-103, 1991. 22.5 µg of membrane proteins are
35 incubated in a mixture of 2Mm of 14C-UDP glucose (specific
activity = 0.34 mCi./mmol, 50 µg of α-amylase, 1Mm of
dithiotreitol (DTT), 1Mm EDTA, 100Mm NaF, 7µM of GTP-γ-S, 1M
of sucrose and 50Mm of Tris-HCL (pH 7.8) in a volume of

100µl. The medium is incubated at 25°C for 1 hour and the reaction is terminated by adding TCA at a final concentration of 5%. The reaction mixture is transferred onto a pre-humidified glass fibre filter. The filter is washed, dried
5 and its radioactivity is counted.

Mulundocandine is used as a positive control.

Control of the vehicle is carried out with the same quantity of 1% DMSO. The results obtained show that in this test the products of the invention show a good activity in
10 particular the products of Example 1.

B - activity on the *Aspergillus fumigatus* enzyme.

The enzyme is prepared according to the process of Beaulieu et al. (Antimicrob. Agents Chemother 38, 937-944, 1994).

15 The protocol used is identical to the protocol described above for the enzyme of *Candida albicans* except that dithiotreitol is not used in the reaction mixture.

In this test the products show a good activity.